Gene cloning and induced expression pattern of IRF4 and IRF10 in the Asian swamp eel (*Monopterus albus*)

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Abstract: The Asian swamp eel (*Monopterus albus*) is one of the most economically important freshwater fish in East Asia, but data on the immune genes of *M. albus* are scarce compared to other commercially important fish. A better understanding of the eel's immune responses may help in developing strategies for disease management, potentially improving yields and mitigating losses. In mammals, interferon regulatory factors (IRFs) play a vital role in both the innate and adaptive immune system; though among teleosts *IRF4* and *IRF10* have seldom been studied. In this study, we characterized *IRF4* and *IRF10* from *M. albus* (*maIRF4* and *maIRF10*) and found that *maIRF4* cDNA consists of 1 716 nucleotides encoding a 451 amino acid (aa) protein, while *maIRF10* consists of 1 744 nucleotides including an open reading frame (ORF) of 1 236 nt encoding 411 aa. The *maIRF10* gene was constitutively expressed at high levels in a variety of tissues, while *maIRF4* showed a very limited expression pattern. Expression of *maIRF4* and *maIRF10* in head kidney, and spleen tissues was significantly up-regulated from 12 h to 48 h post-stimulation with polyinosinic: polycytidylic acid (poly I:C), lipopolysaccharide (LPS) and a common pathogenic bacteria *Aeromonas hydrophila*. These results suggest that IRF4 and IRF10 play roles in immune responses to both viral and bacterial infections in *M. albus*.

Keywords: *Monopterus albus*; IRF4; IRF10; Poly I:C; Up-regulation

Interferon regulatory factors (IRFs) are ancient molecules conserved throughout the evolution of metazoans and play a vital role in the innate and adaptive immune system (Tamura et al, 2008). To date, 11 IRF family members (IRF1−11) have been described in vertebrates and invertebrates (Huang et al, 2010), with IRF1−10 being present in most vertebrate species and IRF11 being found in non-vertebrate deuterostomes (Huang et al, 2010). Phylogenetic analysis of these 11 IRF proteins demonstrated that they can be subdivided into four groups that reflect their evolutionary history (Nehyba et al, 2009; Xu et al, 2010). Previous studies showed that in humans (*Homo sapiens*) and mice (*Mus musculus*), IRF4 was expressed in most types of immune cells, and has critical functions in B cell differentiation and immunoglobulin production (De et al, 2012). Likewise, IRF4 seems to also play important roles in the development and function of T helper cells, regulatory T (Treg) cells, dendritic cells (Xu et al, 2012) and $CD4^+$ T cell differentiation (Suzuki et al, 2004). Other studies on chickens (*Gallus gallus*) found that IRF4 was mainly expressed in the bursa, bursal lymphocytes (Nehyba et al, 2002), and thymus (Dougherty et al, 2009) and it is capable of repressing the expression of ovalbumin gene (Dougherty et al, 2009). ConA can induce the expression of IRF4 in splenic cells, while IFNs cannot induce the expression of IRF4 (Nehyba et al, 2002). Furthermore, among teleost, pathogen-associated molecular patterns (PAMPs) were found to stimulate the IRF4 expression in rainbow trout (*Oncorhynchus mykiss*) (Holland et al,

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2010) and rock bream (*Oplegnathus fasciatus*) (Bathige et al, 2012).

Belonging to the same IRF4 subfamily, IRF10 was previously found to have been eliminated or rendered non-functional in both mice and humans (Nehyba et al, 2009). Curiously though, IRF10 can elevate the expression of major histocompatibility complex (MHC) class I molecules and guanylate-binding protein (GBP) and interfere with the induction of the type I IFN target genes in chickens (Nehyba et al, 2002). Both MHC class I and GBP play important roles in viral infections (Nehyba et al, 2002; Hu et al, 2011). Another feature of IRF10 is that similar to IRF4, it can also repress the expression of ovalbumin gene (Dougherty et al, 2009) and ConA can induce the expression of IRF10 in splenic cells (Nehyba et al, 2002). Furthermore, type I IFN and IFN-γ seem capable of inducing the expression of IRF10 in primary fibroblasts, though the expression of IRF10 is induced relatively late and needs other protein synthesis (Nehyba et al, 2002). In the Japanese flounder (*Paralichthys olivaceus*), bacteria or viral hemorrhagic septicemia virus (VHSV) can increase the expression of IRF10 in kidney tissue (Suzuki et al, 2011), though in zebrafish (*Danio rerio*) IRF10 was found to be a negative regulator of interferon transcription (Li et al, 2013). Clearly the diversity of roles and effects of IRF10 could use some clarification, especially regarding the role IRF10 plays in immune responses following viral or bacterial infections.

In recent years, the Asian swamp eel (*Monopterus albus*) has become one of the most economically important freshwater fish in East Asia. Unfortunately, data on immune genes of *M. albus* are scarce as compared to other commercially important fish. Given the eels economic importance, a clearer understanding of its immune responses may help to develop strategies for disease management, which may potentially aid in aquaculture, increase yields, or decreases loses of this species. In this study, we cloned full-length cDNAs of *M. albus* IRF4 (maIRF4) and maIRF10 and then investigated the tissue distribution of these two genes' expression. Paired with this analysis, we also opted to investigate a common pathogen afflicting the Asian swamp eel, *Aeromonas hydrophila,* the causative agent of Septicemia in this species (Yang et al, 2008; He et al, 2010). Pathogens of some disease such as stigmatosis in Asian swamp eel had not been investigated clearly. For this analysis, we measured the change in maIRF4 and maIRF10 gene expression in the head kidney (HK), spleen (SP), and gills (GI) following stimulation with PAMPs, e.g. polyinosinic: polycytidylic acid (poly I:C) and lipopolysaccharide (LPS), and a common pathogenic bacteria, *A. hydrophila*.

MATERIAL AND METHODS

Fish

Eels of 150−200 g body weight were obtained from the aquaculture base of Yangtze University, China. Eels were maintained in fiberglass tanks supplied with a continuous flow of recirculating freshwater at 24±1 °C Fish were fed once daily on water earthworms, and were acclimated for at least two weeks prior to experimentation.

Gene cloning and sequence analysis

cDNA samples were prepared from head kidney with the first strand cDNA synthesis kit (Fermentas, Canada). First, degenerate primers IRF4-F/IRF4-R and IRF10-F/IRF10-R were designed against conserved region and used for PCR amplification of the internal region of *maIRF4* and *maIRF10* that was obtained from cDNA samples. PCR products were respectively isolated using a Gel Extraction Kit (Tiangen, China), cloned into a pMD18-T vector (TaKaRa, Japan) and transformed into *Escherichia coli* strain DH5α competent cells. Putative clones were then screened *via* PCR using the aforementioned primers, and the selected clones were sequenced. 5′-RACE and 3′-RACE were performed with gene-specific primers and adaptor primers to obtain the full-length cDNA sequence of *maIRF4* and *maIRF10*. Universal primers mix (UPM) was obtained from mixing UPM Long and UPM Short at a ratio of 1:100. For 3′-RACE, PCR was initially performed with primers UPM/3-F1 followed by a nested PCR with primers UPM/3-F2. For 5′-RACE, the adaptor primers AAP and AUAP were used. RNA from *M. albus* spleen was reverse-transcribed at 42 °C using the gene-specific primer 5-R1. Following synthesis of the first strand cDNA, the resulting purified cDNA was used in the TdT-tailing reaction, and the tailed cDNA was then amplified by primers 5-R2 and AAP. A dilution of the original PCR (0.1%) was re-amplified using AUAP and a nested 5-R3 primer (all primers listed in Table 1).

The nucleotide sequences generated were assembled and analyzed with AlignIR (LI-COR, Inc.). Sequence identities were calculated using the DNAStar

Table 1 Oligonucleotide primers used in the study

Name	Sequence (5′–3′)	Usage
UPM Long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	3′-Race PCR universal primers
UPM Short	CTAATACGACTCACTATAGGGC	
AAP	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG	5′-Race PCR universal primers
AUAP	GGCCACGCGTCGACTAGTAC	
IRF4-F	GA(G)AGC(T)CAGCTGGACATCTC	Cloning for *maIRF4* internal fragment
IRF4-R	ACAGGAGCTGCCTGGCA(G)AAC	
IRF4-5-R1	CTCCCGCATCACAG	5′ RACE 1st round PCR
IRF4-5-R2	GAGAAAGGTGAAGGCTGGCTGT	5′ RACE 2nd round PCR
IRF4-5-R3	GGGATGATGCGGTAGACTTTGT	5′ RACE 3rd round PCR
IRF4-3-F1	ACGGAGACAAGCCCAACAAGC	3′ RACE 1st round PCR
IRF4-3-F2	GTCGGCTGCTGCCTCGTTTCC	3′ RACE 2nd round PCR
IRF10-F	GAGCGC(G)AA(G)CCAGCTGGACATC	Cloning for *maIRF10* internal fragment
IRF10-R	CTCC(G)CGCTCCAGC(T)TTGTTGGG	
IRF10-5-R1	CTGGGCTCTTGGTG	5′ RACE 1st round PCR
IRF10-5-R2	GCTCTTGGTGGTCACTTTCATT	5′ RACE 2nd round PCR
IRF10-5-R3	AAGCGGGCTGAAGAAGGTGATA	5′ RACE 3rd round PCR
IRF10-3-F1	AAGTGACCACCAAGAGCCCAGAT	3′ RACE 1st round PCR
IRF10-3-F2	CAATGGCTCGCCTTCTTTGTCA	3′ RACE 2nd round PCR
β-actin F	CAGTCCTCCTAAGGCGATAA	Real-time quantitative PCR control
β-actin R	GCATCATCTCCAGCAAAGC	
IRF4-RT- F	ACGGAGACAAGCCCAACAAG	Real-time quantitative PCR
IRF4-RT -R	ACAAGTGGCTGCCCGTCTG	
IRF10-RT- F	ACAATGGCTCGCCTTCTTT	Real-time quantitative PCR
IRF10-RT- R	TGGGACCACTCCAATACAC	

5.0 (http://www.mega software. net/index.html/). Multiple sequence alignments were generated using Clutal W 2.02. Phylogenetic trees were created by the neighbor-joining method using MEGA 5.01 and were bootstrapped 1,000 times.

Tissue distribution

Intestines (I), HK, trunk kidney (TK), GI, brain (BR), liver (L), SP, muscle (M), gonad (GO), whole blood (BL) and skin (SK) from four fish were collected and used for RNA isolation using TRIzol (Invitrogen, USA) in order to analyze the expression of *maIRF4* and *maIRF10* in healthy eels. Real-time PCR was conducted to detect the expression of *maIRF4* and *maIRF10* in these tissues. The expression of *maIRF4* and *maIRF10* in different tissues was calculated relative to the expression level of β-actin.

Real-time PCR analysis

The *maIRF4*, *maIRF10* and house-keeping gene β-actin cDNA fragments were generated via RT-PCR. A common reference with purified PCR products of the three genes was separately used for quantification. Primers used for detection of gene expression are detailed in Table 1. PCR reactions were performed using Chromo 4™ Continuous Fluorescence Detector (MJ Research). Amplifications were carried out at a final volume of 20 μL containing 1 μL DNA sample, 10 μL 2×SYBR green Real time PCR Master Mix (Toyobo, Japan), 2 μL of each primer and 5 μL H_2O. PCR amplification consisted of 5 min at 95 °C, followed by 40 cycles consisting of 10 s at 94 °C, 20 s at 58 °C, 20 s at 72 °C and read plate at 82 °C. Melting curve analysis of amplification products was performed at the end of each PCR reaction in order to confirm that a single PCR product was detected. Each sample was run in duplicate to ensure accuracy.

Challenge trial using different stimulus

To study the effect of immunostimulants on the expression of *maIRF4* and *maIRF10*, four groups of fish (three fish each group) were respectively injected intraperitoneally (i.p.) with either 500 μL 2 mg poly I:C/mL (Sigma), 500 μL 2 mg LPS/mL (*E. coli* O127:B8, Sigma), 1×10^8 CFU *A. Hydrophila*/mL, or 500 μL PBS to serve as a control. Consecutively, at 0 h, 12 h, 24 h, 48 h after injection, three fish were killed, and tissues from the head kidney, gill and spleen were collected for total RNA extraction and gene expression detection via real-time PCR.

Statistical analysis

Fold change was calculated as (Ts/Tn)/(Cs/Cn) where Ts equals the treated sample assayed for the specific gene and Tn equals the treated sample assayed for the

normalizer gene (β-actin), and Cs and Cn respectively equals the calibrator group with the specific and normalizing gene (Purcell et al, 2004). One way-analysis of variance (ANOVA) and an LSD post hoc test was used to statistically analyze the expression data, with $P<0.05$ being considered statistically significant.

RESULTS

Cloning and characterization of maIRF4 and maIRF10

Analysis showed that *maIRF4* cDNA (GenBank accession no. JX463267) is approximately 1.7 kb in length, with an open reading frame (ORF) of 1 356 nucleotides, encoding a protein of 451 amino acid (aa) residues with a putative molecular weight of 51.7×10^3 and an isoelectric point (*p*I) of 9.61. Furthermore, the *maIRF4* cDNA contains 22 microsatellite dinucleotide (T-G) repeats in the 3′ UTR. The full-length cDNA of maIRF10 meanwhile comprises 1 744 nucleotides (GenBank accession no. JX463268). The ORF encodes a 411 aa protein, with a calculated molecular weight of 58.97×10^3 and a *p*I of 8.85. The 3′-UTR has two ATTTA motifs that mediate mRNA degradation (Iwai et al, 1991) and the polyadenylation signal (AATAAA) lies 27 bp upstream of the polyA tail.

Phylogenetic analysis showed that the IRF4 molecules diverged from the other IRF4 subfamily members (IRF8, 9, 10), with eel IRF4 clustering with the other teleost IRF4s (Figure 1). Furthermore, teleost IRF4 diverged into two clades, with maIRF4 having the highest similarity with medaka IRF4-2 (Figure 1). The putative maIRF4 protein was 45.6% to 84.0 % identical to IRF4 proteins from mammals, chickens, frogs and other fish (Figure 2), while maIRF10 is 43.4% to 76.6% identical to its homologous proteins in mammals, birds, amphibians and other fish (Figure 3). Additionally, flounder IRF10 was the closest to maIRF10. MaIRF4 and maIRF10 had the DNA-binding domain and IRF association domain (Figure 2, Figure 3), both of which were important domains in the IRF family (Lohoff & Mak, 2005).

Tissue expression

Real-time PCR analyses showed that the expression of *maIRF4* in all tested tissues was quite low, with relative abundant in intestine (0.21×10^{-3}-fold), head kidney (0.16×10^{-3}-fold) and trunk kidney (0.14×10^{-3}-fold) (Figure 4). However, constitutive expression of *maIRF10* transcripts was detected in all tissues studied. Compared with β-actin, expression was predominantly in head kidney (7.2×10^{-3}-fold) and whole blood (6.9×10^{-3}-fold). And a relatively low level of maIRF10 expression was detected in brain (1.1×10^{-3}-fold) and spleen (0.4×10^{-3}-fold) (Figure 4).

Induced expression

In order to further investigate the differences pattern, *maIRF4* and *maIRF10* expression induced by poly I:C, LPS and *A. hydrophila* were examined. Stimulation by poly I:C up-regulated *maIRF4* expression at 12 h post-stimulation (hps) in head kidney (23.2-fold), spleen (15.3-fold) and gill (3.8-fold) tissue ($P<0.05$). Subsequently, expression increased, reaching a peak in head kidney (45.8-fold), spleen (27.5-fold) and gill (5.4-fold) at 24 hps ($P<0.05$) (Figure 5). Following LPS stimulation, *maIRF4* mRNA levels were initially increased in head kidney and spleen at 12 hps, and remained elevated at 24 hps before decreasing to basal levels in spleen. In gill only at 24 hps was a significant increase seen. After *A. hydrophila* stimulation, *maIRF4* transcript levels were enhanced in all three tissues studied at all time-points and peaked at 24 hps, with a fold change of 17.7 in head kidney, 13.6 in spleen and 6.51 in gill (Figure 5).

The level of *maIRF10* induction was always lower than that of *maIRF4*. *MaIRF10* expression was significantly up-regulated at all times-points by poly I:C in head kidney and in spleen but not in gills. The *maIRF10* expression level peaked at 24 h in head kidney (5.3-fold) and spleen (5.8-fold). Following LPS stimulation, *maIRF10* mRNA expression increased from 12 hps to 48 hps (up to 3.6-fold) in head kidney, but significant up-regulation occurred at 24 h (3.6-fold) in the spleen, with no significant change in gills. Following *A. hydrophila* stimulation, *maIRF10* transcript levels were also enhanced in head kidney and spleen at 12 and 24 hps, with a peak fold change of 6.3 and 4.9 at 24 hps, respectively. Notably, levels remained elevated to 48 hps in head kidney. No significant changes were detected in *maIRF10* expression in the gills at any time-point following stimulation by *A. hydrophila* (Figure 5).

DISCUSSION

Phylogenetic analysis showed that teleost IRF4 genes aggregated into two branches, suggesting that these two fish IRF groups arose through teleost-wide whole genome duplication (Figure1). However, only one IRF4 gene was cloned in the Asian swamp eel. This may be

explainable by a twice genome duplication in the species while a third genome duplication occurred in other teleost (Zhou et al, 2002). The *maIRF4* gene is highly conserved compared with flounder IRF4 and medaka IRF4-2, respectively exhibiting 84.2% and 83.7% identity over the entire protein. The serine-rich domain is a target for virus induced phosphorylation that facilitates interaction with other IRF members and subsequent activation of virus clearance signaling pathways (Sun et al, 2007). This domain is present in zebrafish IRF4-1, fugu IRF4-1, medaka IRF4 (Figure 2) and rock bream (*O. fasciatus*) (Bathige et al, 2012) but absent from IRF4 in many other species including humans, chickens, xenopus (*Xenopus tropicalis*) (Figure 2), rainbow trout (Holland et al, 2010) and eel, indicating significant evolutionary divergence of IRF4. All IRF10 genes from birds, amphibians and fish are closely clustered and closely related to IRF4 (Figure 1). Compared with these

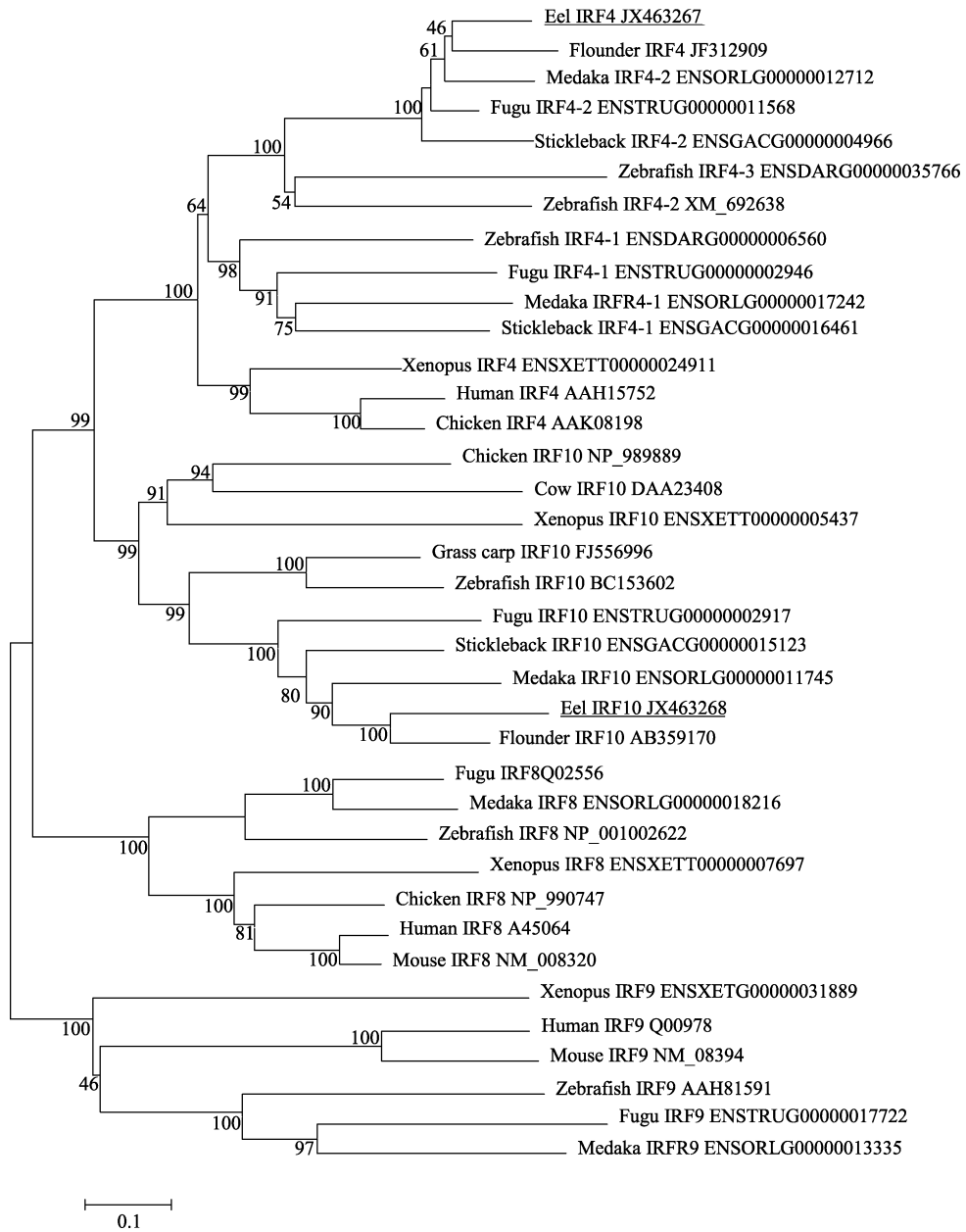

Figure 1 Phylogenetic tree analysis of IRF4, IRF8, IRF9 an IRF10 among vertebrates

A neighbor-joining phylogenetic tree of IRF proteins based on protein sequences analyzed with Clustal W and MEGA 5.0. Data were analyzed using Poisson correction, and gaps were removed by pairwise deletion. The degree of confidence for each branch point was determined by bootstrap analysis (1 000 times). The sequences of IRFs used for the analysis are derived from the GenBank and Ensembl Databases, with accession numbers added after the genes.

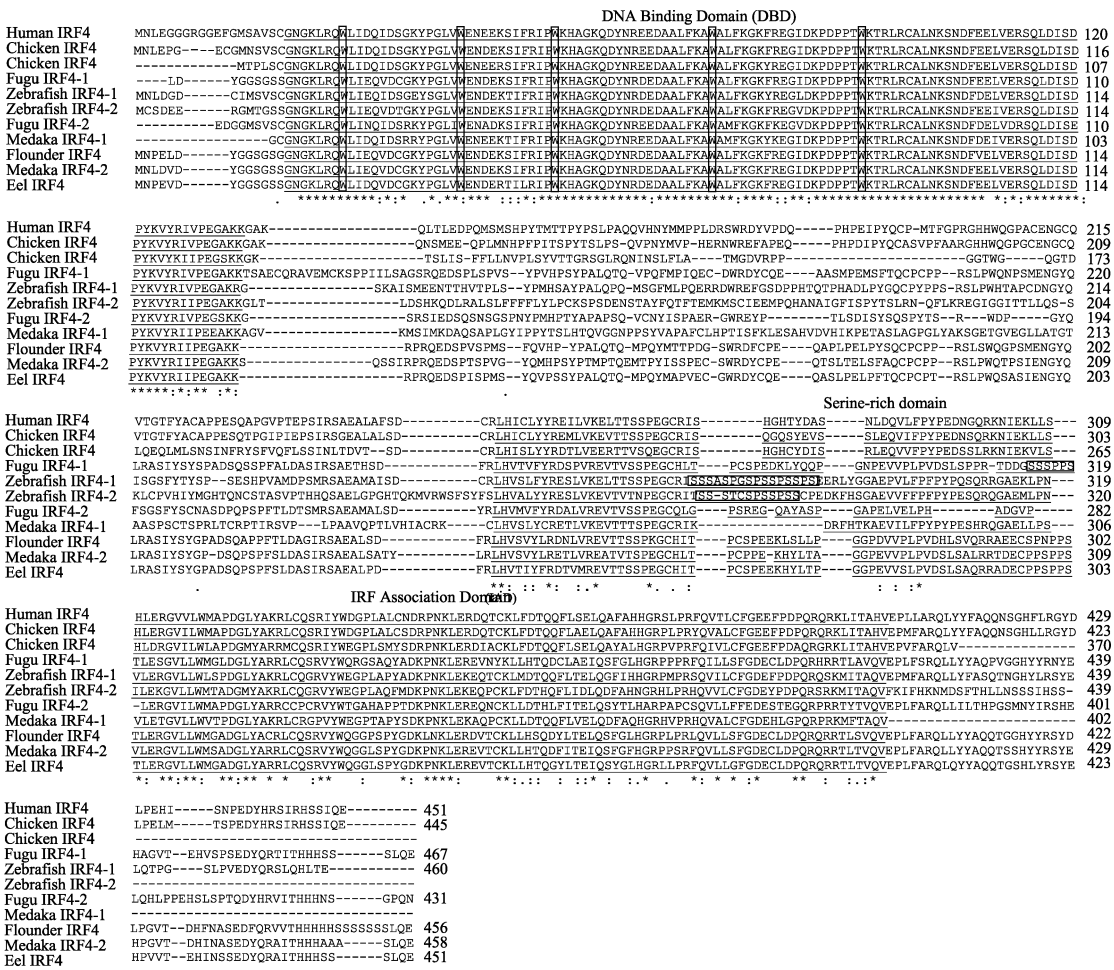

Figure 2 Multiple alignment of maIRF4 amino acid sequence with other IRF4 proteins

Symbol (*) represents identical residues, (:) conservative substitution and (.) similar residues. Missing amino acids are denoted by hyphens. The DNA-binding domain (DBD) and IRF association domain (IAD) are highlighted in grey. The conserved tryptophan (W) residues that comprise a ‘‘tryptophan cluster’’ are boxed. A serine-rich domain is also boxed.

IRF10 family members, the maIRF10 protein sequence showed the greatest homology with Japanese flounder, medaka (*Oryzias latipes*) and stickleback (*Gasterosteus aculeatus*), ranging from 59.6% to 76.6% identity across the whole protein.

Generally, *IRF10* is expressed in all tissues while *IRF4* has a much more limited expression pattern. For example, among chickens *IRF10* is detected in all tissues, while only faint signals of *IRF4* mRNA were detected in all the previously tested tissues in chicken or mice (Dougherty et al, 2009; Takaoka et al, 2008). Consistent with these results, *maIRF10* mRNA was found to be abundant in all the tissues we analyzed, with predominant expression in whole blood, skin and head kidney, while *maIRF4* expression was significantly lower than that of *maIRF10* in all the same tissues (Figure 4). The highest expression of *maIRF4* was detected in the intestine, head kidney and gill, major immune tissues/organs, trunk kidney and brain. A previous study reported that among the Japanese flounder, *IRF10* mRNA was strongly expressed in gill, head kidney, heart, peripheral blood lymphocytes, spleen and trunk kidney (Suzuki et al, 2011), while another study on rainbow trout found the highest expression levels of *IRF4* in lymphomyeloid-rich fish tissues (e.g., spleen, head kidney and gills; Holland et al, 2010). *MaIRF10* also shows relatively high expression in non-immune tissues such as muscle, gonad and brain, indicating that IRF10, as well as IRF4 and IRF8, is present in at least some non-immune tissues (Dadoune et al, 2005; Eguchi et al, 2008).

Previously, it was reported that IFN1, IFN-γ and ConA can up-regulate IRF10 expression (Nehyba et al, 2002). However, induction expression of IRF10 occurs relatively late, and is dependent on protein synthesis.

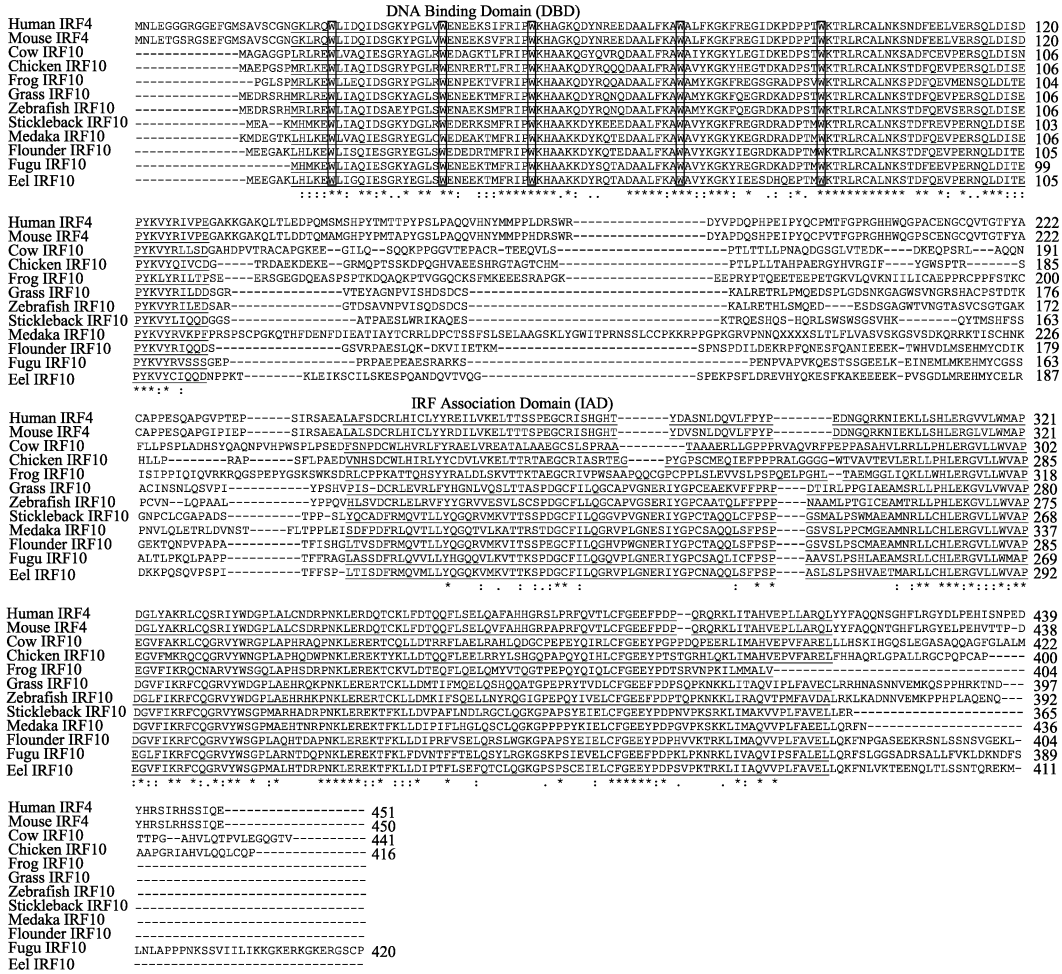

Figure 3 Multiple alignment of maIRF10 amino acid sequence with other IRF10 proteins

Symbol (*) represents identical residues, (:) conservative substitution and (.) similar residues. Missing amino acids are denoted by hyphens. The DNA-binding domain (DBD) and IRF association domain (IAD) are highlighted in grey. The conserved tryptophan (W) residues that comprise a ‘‘tryptophan cluster’’ are boxed.

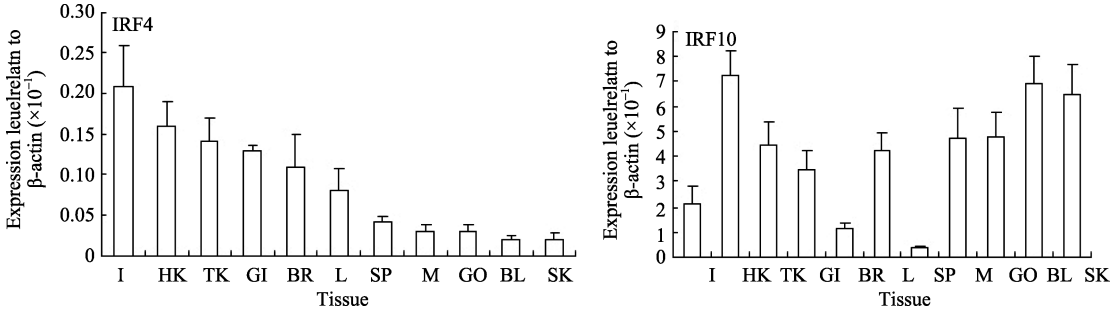

Figure 4 Expression analysis of maIRF4 and maIRF10 in different tissues

Real-time quantitative PCR was performed with cDNA samples prepared from whole blood (BL), brain (BR), gill (GI), skin (SK), muscle (M), intestines (I), spleen (SP), liver (L), gonad (GO), head kidney (HK) and trunk kidney (TK). Bars are averages plus standard deviations of tissue data from 4 fish.

Neither IFN1 or IFN-γ induce IRF4 expression, though IRF4 is known to be induced by ConA, plant lectins, CD3, phorbol-12-myristate-13-acetate (PMA) and IgM cross-linking in chicken and mouse (Ma et al, 2006; Nehyba et al, 2002). In humans, dengue virus and T cell receptor cross-linking has likewise been known to

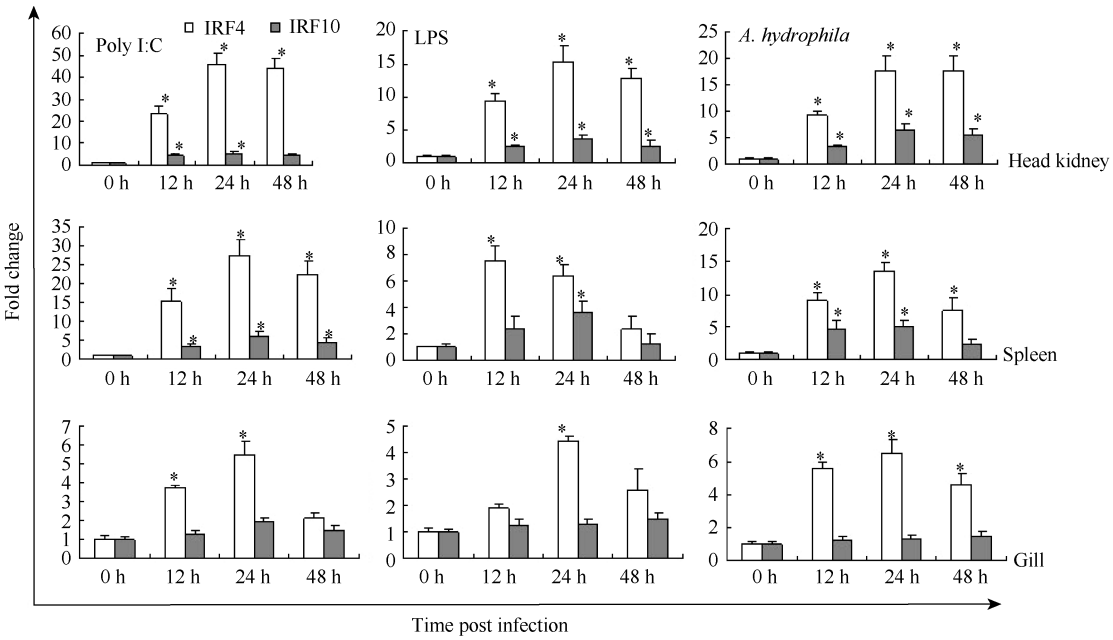

Figure 5 Expression levels of maIRF4 and maIRF10 induced by poly I:C, LPS and *Aeromonas hydrophila* in head kidney, spleen and gill

Fish were injected with each stimulant and 12 h, 24 h and 48 h, and tissues were then isolated for RNA extraction and cDNA synthesis. The transcript levels were obtained using real-time PCR and expressed relative to the 0 h healthy (control) fish and presented as the fold change after being normalized to the expression of β-actin. Bars denote averages plus standard deviations of tissues from 3 fish. * over the bar indicates a significant difference after a treatment by one-way ANOVA (*: $P<0.05$).

increase IRF4 mRNA levels (Chen et al, 2008; Matsuyama et al, 1995). Among the Asian swamp eel, we found that poly I:C, LPS and *A. hydrophila* induced *maIRF4* and *maIRF10* expression, wherein intraperitoneal injection of poly I:C significantly induced *maIRF4* and *maIRF10* expression from 12 hps to 48 hps (Figure 5). Although *maIRF4* and *maIRF10* expression induced by LPS or *A. hydrophila* was lower than that induced by poly I:C, there was a significant increase in expression in both the head kidney and spleen following both treatments (Figure 5). *maIRF4* expression peaked at a 45.8-fold increase in head kidney and *maIRF10* reached a maximal expression level (5.8-fold) at 24 hps following poly I:C stimulation. Similar results have been found in other fish: for example, in rainbow trout, IRF4 was significantly up-regulated by PMA (4-fold above control levels) in splenocytes in vitro (Holland et al, 2010) while in the Japanese flounder, IRF10 expression was up-regulated 7.8-fold at 6 hps by LPS and peaked at a 23.6-fold up-regulation at 6 hps following poly I:C stimulation of peripheral blood leukocytes (Suzuki et al, 2011).

Further comparison of IRF10 and IRF4 inducible expression highlighted a greater increase in *maIRF4* expression as compared with that of *maIRF10*. These results are consistent with previous expression studies conducted on birds (Nehyba et al, 2002), which found that IRF4 constitutively expressed at a low levels in most tissues and organs among chickens (Dougherty et al, 2009) and that inducible expression of IRF10 requires protein synthesis, with IRF10 playing a unique role in the later stages of antiviral defense (Nehyba et al, 2002). By contrast, in rainbow trout, LPS significantly down-regulated IRF4 expression in splenocytes (Holland et al, 2010). Similarly, estrogen was also found to decrease the expression of IRF4 and IRF10 in chickens (Dougherty et al, 2009).

In summary, in this study the complete *maIRF4* and *maIRF10* genes of the Asian swamp eel were cloned for the first time. Analysis of the overall amino acid sequence, identity and phylogenetics, showed that the cDNA encoding *maIRF4* and *maIRF10* exhibited homology with other IRF4 and IRF10 proteins of tetrapod species and fish sequences currently deposited in public databases. Furthermore, *maIRF10* was constitutively expressed at high levels in all the tissues investigated, while the transcript level of *maIRF4* was found to be quite low in all organs. Stimulation by Poly I:C, LPS

and *A. hydrophila* were also shown to induce *maIRF4* and *maIRF10* expression, indicating that these two factors likely play a part in the immune reaction to both bacterial and viral infections.

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